

Evaluation of FloSeal as a Potential Intracavitary Hemostatic Agent

Harold G. Klemcke, PhD

Background: Noncompressible hemorrhage is a major cause of death in combat and civilian trauma. When surgery is unavailable, one potential solution to such hemorrhage might be the introduction of an agent into the closed body cavity to provide hemostasis via a combination of coagulative and tamponade effects. FloSeal is an agent containing collagen and thrombin with proven hemostatic efficacy when applied with manual pressure to a bleeding site. The current studies were conducted to analyze the ability of FloSeal to reduce blood loss and increase survival time when applied directly, immediately, and without additional pressure to a severe liver injury in rats.

Methods: Male rats were anesthe-

tized and catheters were placed in the carotid artery (for measurement of blood pressure) and jugular vein (for resuscitation with lactated Ringers, 3.3 mL/min/kg BW). After midventral laparotomy, the liver was exposed and caudal portions of both medial lobes (~1% of body weight) were rapidly excised. FloSeal (5 mL, 800 units Thrombin/mL) or vehicle (5 mL, 0.9% NaCl) was directly and immediately applied to the cut liver surface. The abdominal cavity was closed and resuscitation initiated. After hemorrhage-induced death, or after euthanasia at 90 minutes, fluid loss (blood + resuscitation fluid) was measured.

Results: Compared with the control group, direct and immediate application

of FloSeal was associated with a reduction in the amounts of fluid lost into the abdominal cavity ($p < 0.01$) (19.2 ± 1.5 versus 25.1 ± 1.5 g) and enhanced mean arterial pressure at 5, 20, and 30 minutes after injury ($p = 0.02$), but neither survival time ($p = 0.12$) nor percent survival ($p = 0.17$) differed between treated and control groups.

Conclusions: Reductions in fluid loss after liver injury and hemorrhage in FloSeal-treated rats in the absence of additional applied pressure are encouraging, and provide evidence for the ability of FloSeal to reduce blood loss when applied immediately and directly to a bleeding tissue.

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Hemorrhage is the leading cause of death from wounds on the battlefield, accounting for over 50% of deaths.¹ It is the second leading cause of death in civilian trauma.² Approximately 80% of hemorrhagic combat deaths are from wounds that are not compressible (inaccessible for manual pressure), and thereby are currently treatable only with surgical interventions. Indeed, in Vietnam up to 90% of all hemorrhagic deaths were from truncal injury.¹ Hence, pre-hospital procedures for reducing or stopping blood loss from noncompressible truncal injuries would greatly reduce combat mortalities. The most common immediate cause of death caused by abdominal wounds is liver hemorrhage,³ and in trauma the liver is the most common solid organ to be damaged.⁴ Interest exists for a hemostatic agent that could be

introduced into a closed body cavity, spread throughout the cavity, and stop bleeding at sites that could only otherwise be reached surgically.⁵ In rats with an open abdominal cavity, a fibrin sealant foam was sprayed directly onto the cut surface of a severe liver injury and reduced blood loss when compared with a placebo foam.⁵

FloSeal (Baxter International Inc., Deerfield, Ill.) is a proprietary combination of specially engineered collagen-derived particles and bovine thrombin. When exposed to blood, FloSeal gelatin granules expand by ~20% to provide a certain amount of tamponade,^{6,7} and thrombin converts fibrinogen to fibrin, which is a requisite component of the blood clot.⁸ FloSeal has been used in humans to reduce bleeding in a variety of surgical situations, including venous and arterial vascular surgery,^{7,9} cardiac valve replacement or cardiopulmonary bypass grafting,⁶ partial nephrectomies,¹⁰ nephrolithotomy,¹¹ endoscopic sinus surgery¹² and transphenoidal pituitary surgery.¹³ However, in a highly controlled study with end-to-end anastomosis of transected abdominal aorta in rabbits, FloSeal with gentle pressure did not significantly decrease blood loss.¹⁴

The ultimate aim of our research is to select a hemostatic material that can be injected through the abdominal wall to reduce or eliminate blood loss from internal injuries without any additional adverse effects on the individual. Ultimately, a hemostatic product is needed that can be applied in a pre-hospital combat area. This would reduce or stop hemorrhage until such time as the patient could be transported to a

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From the U.S. Army Institute of Surgical Research, Fort Sam, Houston, Texas.

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Address for reprints: Harold G. Klemcke, U.S. Army Institute of Surgical Research, 3400 Rawley E. Chambers Avenue, Fort Sam, Houston, TX 78234-6315; email: harold.klemcke@amedd.army.mil.

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Table 1 Measures and responses in preliminary study to test effects of liver ligation and/or clamping on blood loss and survival

| Measure | Mean \pm SEM |
|--------------------------------------|-----------------|
| | (n = 6) |
| Body weight (grams) | 443 \pm 20 |
| Cut median liver lobe weight (grams) | 4.6 \pm 0.3 |
| Cut liver weight/body weight (grams) | 1.04 \pm 0.05 |
| Fluid loss (grams) | 8.93 \pm 2.57 |
| Resuscitation fluid volume (mL) | 40.4 \pm 11.5 |
| Survival time (min) | 55.7 \pm 4.3 |
| Percent survival for 60 min (%) | 83.3 |

SEM, standard error of the mean.

hospital environment. The current study was conducted to evaluate the potential use of FloSeal as such an intracavitary hemostatic agent by first testing its ability to reduce blood loss and increase survival after direct application to a liver injury with the abdomen open during application and without additional compression. Such a study represents an initial and essential first phase in the evaluation of any potential intracavitary hemostatic agent.

MATERIALS AND METHODS

Male out-bred Sprague Dawley rats (~400 g) were housed singly in plastic cages under controlled temperature (25°C) and humidity, with food and water constantly available. Rats were randomly assigned to treated or control groups. All rats received care in strict compliance with the 1996 *Guide for the Care and Use of Laboratory Animals*, published by the National Research Council. The protocol was approved by the USAISR/BAMC Institutional Animal Care and Use Committee. Rats were anesthetized initially with 5% isoflurane in 100% oxygen, and maintained under 2% isoflurane for a surgical plane of anesthesia. Catheters (Intramedic, Clay Adams Polyethylene Tubing, PE 50, ID 0.58 mm, OD, 0.965 mm [Becton Dickinson, Sparks, MD]) were surgically implanted in the carotid artery (for blood sample removal and for measures of heart rate and blood pressure) and in the jugular vein (for fluid resuscitation using

lactated Ringers, 3.3 mL/min/kg). Then, a 2-mL blood sample was removed using a 2.9-mL S-Monovette containing sodium citrate (Sarstedt, Newton, NC) and used for assessing blood coagulation measures (see below). Body temperature was monitored throughout all procedures and maintained close to 37°C through the use of heating pads and an overhead heat lamp. The abdominal cavity was opened via a midventral laparotomy, and the liver was exposed. Using a small cautery, four marks were made on the ventral surface of the left and right medial lobes of the liver, 1.3 cm from the entry of the inferior vena cava into the diaphragm. A scissors was used to cut rapidly along these marks, and the medial lobes distal to the marks were excised. The cut portion of the liver was removed from the body cavity, and 5 mL of FloSeal or vehicle (0.9% sterile saline) were immediately applied directly to the cut liver surface. The abdominal cavity was then closed and fluid resuscitation was initiated. Heart rate, blood pressure, and rectal temperature were recorded at 5-sec intervals for 90 minutes or until the rat died.

After 90 minutes, any surviving rats were killed with intravenous sodium pentobarbital (150 mg/kg). After death, the abdominal cavity was reopened and fluid (blood + blood clots + resuscitation fluid) present in the abdominal cavity was collected with pre-weighed absorbent gauze. Subsequent weighing and subtraction of the weight of FloSeal or saline added to the abdominal cavity provided the weight of fluid accumulated in the abdominal cavity because of the injury and associated hemorrhage.

After death, the residual medial liver lobes remaining in the abdominal cavity were removed, weighed, and placed in 0.9% saline. Subsequently, this liver section was dissected until a section ~3 mm thick remained, one side of which represented the cut surface associated with the original liver cut that produced the traumatic hemorrhage. This liver section was then placed in a Plexiglass holder, and the original cut surface photographed with a Kodak DCS 760 Digital camera with a Nikkor 60-mm lens. The digital image was then analyzed using the National Institutes of Health program Image 1.60 to determine the area of the cut surface. This measure was conducted three times for each liver, and the

Table 2 Comparison of injury severity, hematological measures, and coagulation measures between control rats and treated rats

| Measure | Treatment | | |
|---|---------------------|---------------------|-------------|
| | Control (n = 16) | FloSeal (n = 15) | Probability |
| Body weight (grams) | 418 \pm 5.0 | 421 \pm 4.5 | $p = 0.68$ |
| Cut median liver lobe weight (grams) | 3.2 \pm 0.3 | 3.2 \pm 0.2 | $p = 0.96$ |
| Cut liver weight/body weight (grams) | 0.74 \pm 0.06 | 0.76 \pm 0.05 | $p = 0.87$ |
| Cut liver surface area (cm ²) | 2.03 \pm 0.09 | 2.31 \pm 0.13 | $p = 0.08$ |
| Hematocrit | 36 \pm 1.9 | 37.8 \pm 0.5 | $p = 0.39$ |
| Hemoglobin (g/dl) | 12.4 \pm 0.7 | 13.2 \pm 0.2 | $p = 0.30$ |
| Platelets (10 ³ /mm ³) | 494 \pm 24 | 519 \pm 18 | $p = 0.42$ |
| Prothrombin time (sec) | 16.9 \pm 0.4 | 17.7 \pm 1.0 | $p = 0.45$ |
| Fibrinogen concentration (mg/dL) | 161.5 \pm 3.6 | 156.2 \pm 4.4 | $p = 0.37$ |

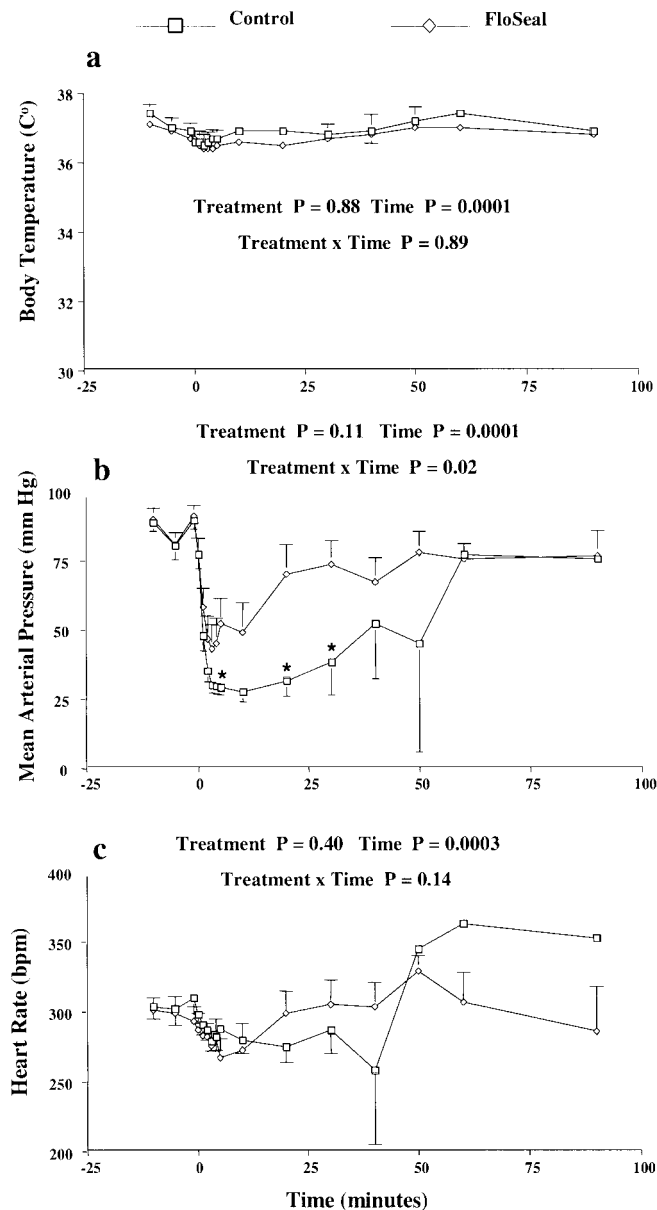


Fig. 1. Body temperature (A), mean arterial pressure (B), and heart rate (C) in rats at various time intervals before (minus relative to time of liver injury) and after (plus relative to time of liver injury) liver injury (time 0). Measures were conducted at 10, 5, and 1 minute before liver injury and at 0, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, and 90 minutes after injury. Data represent mean \pm standard error of the mean of the number of surviving rats at each time point. The number of rats contributing to observed data for controls was: at -10 to +2 minutes, $n = 16$; at +3 to +5 minutes, $n = 15$; at +10 minutes, $n = 13$; at +20 minutes, $n = 11$; at +30 minutes, $n = 5$; at +40 minutes, $n = 3$; at +50 minutes, $n = 2$, and at +60 to 90 minutes, $n = 1$. The number of rats contributing to observed data for FloSeal treatment was: at -10 to +4 minutes, $n = 15$; at +5 minutes, $n = 14$; at +10 minutes, $n = 11$; at +20 minutes, $n = 7$; at +30 to +40 minutes, $n = 6$; at +50 to +60 minutes, $n = 4$; and at +90 minutes, $n = 3$. * indicates differences between treated and control rats at that time interval ($p < 0.05$).

mean of these three determinations was used for subsequent statistical analysis. This measure plus the weight of the cut medial lobes divided by the body weight were used as measures of the severity of the liver injury.

FloSeal was prepared according to the manufacturer's directions. Briefly, 5 mL of sterile saline was added to the bovine thrombin (5000 US units of total activity). After thorough mixing, 4 mL of this thrombin solution was withdrawn into an empty 5-mL syringe provided by the manufacturer and, through the use of a Luer connector assembly, this 4-mL thrombin solution was transferred into a second syringe containing Gelatin Matrix. This mixture was transferred between syringes a total of 20 times to ensure adequate mixing. Preparation was conducted ~ 10 minutes before application. The entire contents of the syringe (5 mL) were used in one continuous application. This quantity was chosen because it constituted that provided by one kit, completely covered the exposed liver surface, and exceeded the average amount (3.41 mL) necessary to successfully treat patients undergoing vascular surgery.⁸

The blood sample removed before liver injury was used to measure complete blood counts, hematocrit, fibrinogen, and prothrombin time. Complete blood counts and hematocrit measurements were conducted on the PENTRA 120 (ABX-Diagnostics, Montpellier, France). Prothrombin time and fibrinogen concentrations were analyzed in plasma at 37°C using the ACL Futura Coagulation System according to the manufacturer's instructions (Instrumentation Laboratory, Lexington, MA).

A preliminary study was designed to ascertain if the injury used in the primary study was survivable for 1 hour if complete or near-complete hemostasis were achieved. Hence, using the same animal model described above, two techniques were used (separately or together) immediately after liver injury to stop blood flow from the cut liver. The first was ligation of the cut liver lobe at its base using umbilical tape (0.25 inches wide). The second was clamping the base of the medial liver lobe proximal to the cut with one or two DeBakey Pediatric multipurpose clamps (5.5 inches). The objective was to use all procedures necessary to stop visible blood loss and allow for a 1-hour survival period. Rats alive at 1 hour were killed, and blood loss was measured as noted above.

Data were analyzed using the Statistical Analysis System (SAS [SAS Institute, Cary, NC]) statistical package.¹⁵ Survival data were analyzed using PROC FREQ and associated Fisher's exact test. Analysis of all survival time data were conducted using the PROC LIFETEST procedure of SAS with associated log-rank nonparametric test. Blood loss, severity scores, and other single point measures were analyzed using one-way analysis of variance (ANOVA) with the main factor of treatment (PROC GLM). Analysis of covariance also was used to assess the appropriateness of various measures (injury severity and preinjury mean arterial pressure [MAP]) to be used as covariates for adjusting other variables

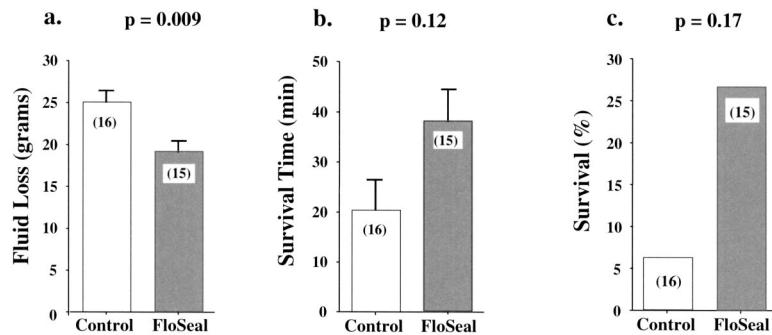


Fig. 2. Effects of immediate and direct application of 5 mL saline (control) or FloSeal to rat cut liver surface on fluid (blood + blood clots + resuscitation fluid) loss into the abdominal cavity (a); survival time (b); and percent of rats surviving a 90-minute observation period (c). Each bar represents the mean \pm standard error of the mean of the number of rats shown in parentheses within the bar.

(e.g., survival time and blood loss). If the covariate was found to be insignificant, it was dropped from the SAS model. Measures taken in each rat at multiple time points (body temperature, MAP, heart rate) were analyzed using two-way repeated measures ANOVA (PROC MIXED; with rat within treatment as a random variable). Means separation tests were conducted using robust, orthogonal contrasts and the conservative *t* test with Bonferroni adjustment for multiple comparisons. All data were tested for homogeneity of variance (PROC ANOVA with associated Levene's test) and normality of distribution (PROC Univariate Normal with associated Kolmogorov-Smirnov test). Data were transformed where necessary to meet assumptions of ANOVA. All data are presented as arithmetic mean \pm standard error of the mean.

RESULTS

In the preliminary study, 5 of the 6 rats used survived the entire 60 minutes (Table 1) with an average fluid loss of less than 10 mL. In the primary study, body weight, cut liver weight, cut liver weight expressed as a percentage of body weight, cut liver surface area, hematological measures, and coagulation measures did not differ between control and FloSeal-treated groups (Table 2). Body temperature did not differ between the two treatment groups, but a 0.61°C decrease ($p = 0.0001$) occurred in both groups in the 10-minute interval before liver injury (Fig. 1A). MAP decreased rapidly in both groups after liver injury to a nadir at 3 minutes (Fig. 1B). At 5, 20, and 30 minutes after injury, MAP was elevated in FloSeal-treated rats compared with control rats ($p \leq 0.02$). Heart rate (Fig. 1C) was unaffected by treatment ($p = 0.40$), achieved a numerical nadir at 3 minutes after liver injury, then gradually increased between 3 and 50 minutes after injury in surviving rats ($p = 0.005$). There were subsequently no time-related changes in heart rate ($p = 0.24$). As determined by analysis of covariance, fluid accumulating in the abdominal cavity was not related to the weight of the cut liver section, body weight, area of the cut liver surface, or fluid resuscitation volume ($p > 0.11$), but it was reduced by the application of FloSeal ($p < 0.01$) (Fig. 2A). However, despite large numerical differences, neither survival time ($p =$

0.12) (Fig. 2B and Fig. 3) nor percent survival ($p = 0.17$) (Fig. 2C) differed between the two groups. Volume of resuscitation fluid used was linearly related to body weight ($p = 0.01$) and the weight of the cut liver fragment ($p = 0.04$), but, even when these were used as covariates, there were no differences ($p = 0.30$) in resuscitation volumes used in control (27.2 ± 7.9 mL) versus FloSeal-treated rats (48.4 ± 8.2 mL).

DISCUSSION

The positive control study clearly demonstrated that the severe liver injury and resuscitation procedures used in the current studies were survivable during the observation period with minimal blood loss if mechanisms that rapidly reduce or stop blood loss were used immediately after injury. In the primary study, rats either died before 1 hour or lived the entire 90 minutes. Hence, even though rats receiving clamps and ligatures to stop bleeding were only observed for 1 hour before killing, it is probable that they would have survived 90 minutes had they been allowed to do so. Immediate application of FloSeal directly onto the cut liver surface without any additional compression did reduce, but did not stop, blood loss. Unlike use of ligation and clamps, this reduced blood loss with FloSeal did not enhance survival time or percent

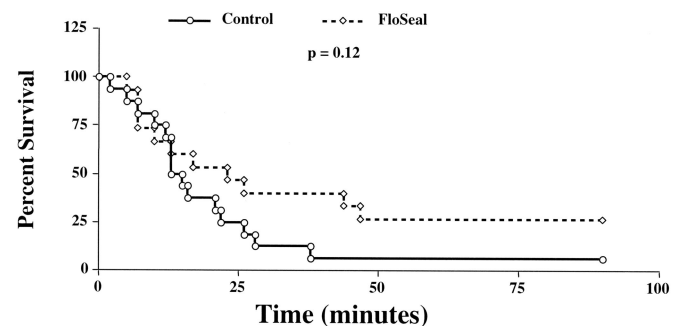


Fig. 3. Kaplan-Meier survival graph for survival time of rats after severe liver injury. Data were compared statistically via use of the SAS program PROC Lifetest and its associated log-rank nonparametric test.

survival. Because of a large variability among animals within treatment groups (survival times ranged from 2 to 90 minutes), and the requisite appropriate use of less robust non-parametric statistics (threefold and 82% numerical differences in percent survival and survival time, respectively), were not statistically significant. Indeed, it is not uncommon for a dichotomy to occur between blood loss and survival time.^{16–18} Such results suggest that differences in blood loss were insufficient to alter survival time or percent survival, and/or that factors in addition to total blood loss are involved in survival, e.g., rate of blood loss or differences in respiration rate or arterial potassium.¹⁹ These results are, however, very relevant because they demonstrate, for the first time in a controlled study, the ability of FloSeal to transiently enhance MAP and to reduce blood loss from injured liver tissue with mixed venous, arterial, capillary, and sinusoidal hemorrhage in the absence of additional compression. Such reductions in blood loss are comparable to those observed with application of a fibrin sealant foam in the same animal model.⁵

A complete sequential evaluation of potential intracavitary hemostatic agents such as FloSeal involves an initial direct application test as performed in the current study. If successful, subsequent tests would examine the ability of the agent to reduce blood loss and enhance survival when applied into a closed cavity and at a site distal to the injury. Indeed, to most closely reflect a battlefield situation, the injection of this agent should be delayed for a certain time interval after creation of the injury, since it is very unlikely that any medical aid would be available immediately after wounding. Preliminary data indicate that, with the current injury model, a 5-minute delay is excessive. Hence, other less rigorous models need to be developed to complete assessments of potential intracavitary agents in a closed cavity with delayed application.

The concept of an intracavitary hemostatic agent such as foam is quite new and appealing.⁵ However, multiple technical problems that need to be overcome are associated with implementation of such a novel procedure. For example, contact between the putative hemostatic agent and the cut tissue surface is made difficult by ongoing hemorrhage and pooled blood. Hence, additional studies are required to provide greater knowledge to improve such mechanisms to treat noncompressible hemorrhage.

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